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PUBLIC HEALTH REPORTS

VOL. 32 JULY 27, 1917 No. 30

DYSENTERY—ARKANSAS-MISSOURI.

Passed Asst. Surg. Ridlon reported July 22 that the dysentery outbreak in northeastern Arkansas and southeastern Missouri was subsiding. It will be difficult to ascertain how extensive the outbreak has been, as the disease is not notifiable, and most of the physicians do not keep case records, nor is the registration of deaths such as to give any useful information. The undertakers' records are also of little value in this connection.

The disease has been widespread in southeastern Missouri and northeastern Arkansas and probably few communities in this area have escaped. Statements of physicians indicate that the disease has also existed in near-by territory in Tennessee and Mississippi. It seems to be the experience of physicians that similar outbreaks of enterocolitis are of annual occurrence, usually beginning in May and extending into June. The outbreak this year is believed to be more severe than usual. Certain physicians in Dunklin County estimate that there have been several hundred cases, with probably 50 deaths, in the county. Measures have been taken to ascertain definitely the specific organism causing the outbreak.

MENINGOCOCCUS CARRIERS.

THEIR RECOGNITION AND TREATMENT.

The "meningococcus carrier" is an individual who harbors the meningococcus without exhibiting symptoms of meningitis. He is of importance to us at this time because of the prospective concentration in camps of a large number of young adults of a susceptible age. These young men are brought into intimate contact with one another and furnish ready material under conditions which have been shown to be favorable for the spread of epidemics of meningitis. The nasopharynx is the location in which the meningococcus is most frequently found, and is the site affected; a fact which is of practical importance in the dissemination of the organism. Here it flourishes, sometimes in great numbers, and probably is expelled by talking, sneezing, or coughing, or is mixed with the secretions of the nose and implanted, by indirect contact, on the mucous membrane of another individual.

Several careful analyses conducted during an epidemic and for a period of two or more years in the absence of an epidemic, have shown that meningococcus carriers are of widespread distribution among the general population, the numbers varying from 1 to 13 per cent of those examined. The higher of these figures has been found in the examination of troops quartered in barracks, except in one study to be referred to later. These analyses exclude those in whom there was a history of contact with a case of meningitis.

"Carriers" are of three general types: Individuals convalescent from an attack of epidemic cerebrospinal meningitis, or posterior basilar meningitis; "contacts" with a case of the disease; and persons who can not be shown to have had any previous contact with the disease. The second and third groups are frequently classed as one. These groups may be subdivided into temporary and persistent, or "chronic," carriers, dependent upon whether they exhibit cultures of meningococci for from a few days to three weeks, or for several weeks or months.

During the first week of convalescence the meningococcus is present in the nasopharynx of the patient in from 60 to 80 per cent of the cases, the number of those carrying the organism progressively decreasing with the convalescence; but in some series of cases there have been as many as 6 per cent positive after two months.

In convalescent cases the meningococcus in the nasopharynx is usually not accompanied by many contaminating organisms, and yields nearly pure cultures. In contacts, however, the number of meningococcus colonies developing from a swab varies, from two or three to a plate to an approximately pure culture.

The recognition of meningococcus carriers is difficult, chiefly because of the parasitic nature of the organism, and because of its very great sensitiveness to drying. Hence the technique adopted should provide for a rapid transfer of the material collected from the patient or contact to a medium which is moist, at approximately body temperature, and containing plasma or other animal fluid.

The collection of material from the nasopharynx is best accomplished with a sterile cotton swab, mounted on a piece of iron wire, which is bent for a half to three-quarters of an inch, at the swab end, through an angle of approximately 45°.

The swab is carried horizontally through the mouth to the pharynx, avoiding the buccal surfaces and the tongue; upon reaching the pharynx the swab is tilted upward, rotated across and against the posterior nasopharynx, and brought out through the mouth in a horizontal position, but with the bent part pointing in the opposite direction to that in which it entered.

The buccal surfaces and secretions should be avoided because of the numerous contaminating organisms, many of which seem to be antagonistic to the growth of the meningococcus. The saliva also, probably, has an inhibitory effect on the meningococcus growth.

The avoidance of contamination from the mouth may be accomplished by the use of a West swab. This consists of a swab made as above, using a pliable wire, inserted in a metal or glass tube of about one-quarter inch diameter, bent as indicated for the swab. This tube shield is plugged with cotton, sterilized in dry heat or in the autoclave, and used by withdrawing the plugs, introducing through the mouth as above described, and when it reaches the pharynx, pushing the swab out past its end to make the sweep over the posterior nasopharynx, and drawing it back into the tube before withdrawing through the mouth.

Phonation by the patient frequently aids in making the swab of the posterior nasopharynx. The area swabbed should be the same as that covered by the curette in the removal of adenoid vegetations of the nasopharynx.

The material collected should be planted immediately on the media on which the organism is to be grown. The time elapsing between the collection of material and its implantation on media affects the results; the longer the time the poorer the results in obtaining cultures; even though the swabs be kept moist, there is a marked falling off in the success of cultivating the meningococcus.

The medium on which the material is planted should, preferably, be at body temperature, or but slightly below it, and should be incubated promptly at 35°–37° C. after sowing.

The medium should include some animal fluid, such as ascitic fluid, hydrocele fluid, blood serum, or laked blood. In the absence of such fluids, the addition of an extract of peaflour serves well.

A medium which has been found very satisfactory is made as follows:

To five parts of a nutrient, 2 per cent neutral agar, made of beef infusion, with 1 per cent glucose, is added at 50°–52° C. one part of sheep serum water (sheep serum, 1; water, 2). Neutrality is tested to phenolpthalein.

The glucose agar is sterilized for one and a half hours in streaming steam. The sheep serum water is autoclaved for 15 minutes with 15 pounds pressure. The mixture of serum water and agar is made shortly before using, with aseptic technique. The tubes of the finished medium should be incubated previous to use, and should be kept moist at all times.

This medium is comparatively clear, and permits easy recognition of the colonies on the plate. It is well adapted to the growth of the meningococcus, so that visible colonies develop with six to eight hours' incubation, and colonies develop to two to three millimeter size overnight.

In the absence of sheep serum, an excellent medium can be made by the addition of laked rabbit, or human, blood to the agar above described. The blood is laked in distilled water, 1 part of blood to 4 of water, and added to the melted agar at 45° to 50° C., in the proportion of 1 part of laked blood in 6 to 10 parts of the agar. Rabbit-blood agar (1 part of blood in 20 of agar) affords a good growth, but has the disadvantage of being opaque, and masking the color of the colony as well as its structure.

The various serum agars are made by the addition of the animal fluid to the agar, in the proportion of from 1 to 5 to 1 to 20.

The English workers exhausted their supply of Witte's peptone, and, finding it difficult to get animal fluids, improvised a medium containing an extract of peaflour, added to a trypsinized meat agar.

The method of its manufacture, as given by Gordon, Hine, and Flack,[1] is as follows:

Formula for Trypagar.

1. PEAFLOUR EXTRACT.

Take 100 grams of peaflour and add 1 liter of distilled water with 100 grams of salt. Mix and steam for one-half hour, stirring constantly. Allow to settle and filter, then sterilize and label "saline pea extract." This peaflour extract should preferably be freshly made for each batch of agar.

2. TRYPSIN BROTH.

Take some fresh bullock's heart, free from fat and vessels, mince the meat very finely and weigh. To each one-half kilo add 1 liter of water and make faintly alkaline to litmus with 20 per cent KOH solution. Heat this slowly to 75° to 80° C. for 5 minutes. Cool to 37° C., add 1 per cent of liquor trypsinæ comp., and keep it at 37° C. for 2½ to 3 hours. When trypsinizing is finished, test for peptone with copper sulphate and KOH, as below, then render slightly acid with glacial acetic acid, and bring slowly to the boil for one-fourth hour. Leave overnight in a cool place, and siphon off the clear liquid in the morning. Make faintly alkiline to litmus, and sterilize in an autoclave at 118° C. for 1 hour on each of 2 days (if not to be used at once).

TO MAKE TRYPAGAR.

Take a measured quantity of the trypsinized broth, add 2 per cent of agar fiber, and 0.125 grams of calcium chloride per liter. Autoclave at 118° C. for three-fourths hour to dissolve the agar. Mix together in a saucepan; titrate with N/10 KOH to give an absolutely neutral reaction. Cool to 60° C., add white of two eggs beaten up with the crushed shells, autoclave again at 118° C. for 75 minutes (or in the steamer for 2 hours).

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[1] Gordon, Hine, and Flack. 1916. Brit. Med. Jour. No. 2916, p. 678.

Filter, add to the filtrate 5 per cent of the sterile pea extract, and sterilize in the ordinary way.

BIURET REACTION FOR PEPTONE.

Take 5 cubic centimeters of broth, add 0.1 cubic centimeter of 5 per cent solution of $CuSO_4$, mix, and then add 5 cubic centimeters of normal NaOH. A true pink color indicates that trypsinization is sufficient; a blue-purple shade that it is incomplete.

PREPARATION OF FIBER AGAR.

Weigh out the required quantity, cut up small with scissors, place in a flask, and wash quickly, twice, in water. Drain thoroughly; add water just to cover, and put in glacial acetic acid, 2.5 cubic centimeters per liter of water. Mix thoroughly and leave for one-fourth hour. Pour off the liquid and wash thoroughly, four or five times, to make sure that all the acetic acid is washed out. Drain carefully and use as above.

Having grown the colonies overnight, they can be recognized by their lack of color, translucency, regularity of contour, uniformly granular structure, and ease of homogeneous suspension in salt solution.

The organism is Gram negative, exhibiting usually many "ghost," or "involution," forms in a young culture; the diplococcus form predominates, this arrangement showing even when fished from solid cultures.

The further identification of the organism can be divided into two stages—that for the immediate detention of a suspect carrier, and that for the definite identification of a carrier.

The first is accomplished by making an agglutination test on an 8 to 18 hour culture with a polyvalent antimeningococcus serum, controlled always in equal titer, with a normal serum, from an animal of the same species as that producing the antimeningococcus serum. The suspension of the meningococci should be sufficiently heavy to facilitate the reading of the test. A turbidity in the final dilution should, as a minimum, be equivalent to that of the typhoid vaccine made by the Army Medical School or the Hygienic Laboratory of the Public Health Service. This is equivalent to 500 parts per million of silica, made in accordance with the standard methods for turbidity determination in water analysis. This turbidity is usually obtained by suspending the overnight growth of a serum agar slant, seeded over the entire surface, in 2 to 5 cubic centimeters of saline solution, and using equal parts of the saline suspension and serum dilution to be tested. The agglutination should be carried out at 55° C. for a period of 12 to 20 hours.

The polyvalent serum should have a titer of at least 1 in 300 for control meningococci of various types.

Agglutination with the antimeningococcic serum in titer of 1 to 100, and the failure of agglutination in the normal serum at half this titer, are sufficient evidence on which to detain an individual as a carrier; but proof of the identity of the organism must be sought in order to avoid detaining those who are not carriers, since the agglutination test on meningococci is irregular and is not specific.

The second stage of identification is accomplished by fermentation tests on sugars.

A solid medium, such as the agar above described, but with a reaction of +0.2 to +0.5 and without the glucose, to which enough pure, sterile litmus solution is added to give a blue tint, serves as the most rapid differential base. The sugars to be selected are glucose, maltose, and mannose. The 10 per cent solutions of each of these should be sterilized in distilled water in the autoclave at 15 pounds pressure for 15 minutes (or better by heating in the Arnold sterilizer for half an hour at 100° C. on three successive days) and 1 cubic centimeter of the solution is then added to 10 cubic centimeters of the agar.

The reaction of the Gram negative organisms common to the nasopharynx is as follows:

[+ =Acid; − =No acid.]

	Glucose.	Maltose.	Mannose.		Glucose.	Maltose.	Mannose.
M. pharyngis siccus	+	+	+	*M. flavus* III	+	+	+
M. flavus I	+	+	+	Meningococcus	+	+	−
M. flavus II	+	+	+	*M. catarrhalis*	−	−	−

Stock cultures of the meningococcus must be transferred every 48 hours in using serum media; but a 1 per cent cornstarch nutrient agar, of neutral reaction, sterilized for 15 minutes in the autoclave at 15 pounds pressure will preserve the organism at the top of a stab culture for a longer time, in event further work in differentiation is desired.

In the present state of our knowledge, the examination of all probable contacts with carriers or patients should be made. However, we do not know just what the value of this procedure is.

Mayer, Waldman, and Gruber[1] examined 9,000 men in barracks, when the garrisons were free from the disease, and found that the number of carriers in the different contingents varied from 2 to 8 per cent. Reexaminations of the various contingents showed more carriers in toto each time, but with wide fluctuations in the individual contingents. In one regiment, 1,900 men were examined during an epidemic, and the carriers isolated. This measure seemed to have no effect on the number of carriers or on the spread of the epidemic.

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[1] Mayer, Waldman, Fürst & Gruber. 1910. Münch. Med. Wchnschr. Vol. LVIII, p. 1584.

W. M. Scott[1] found that 13.7 per cent of 138 noncontacts, in an outpatient dispensary, carried organisms similar to meningococci. (Scott's agglutination tests were not controlled by normal serum.) On the other hand, there is circumstantial evidence to the effect that carriers are responsible for the dissemination of the disease, and that they in turn may develop the disease.

The treatment of such carriers is by isolation from contact with others in quarters, in the mess hall particularly, and in gatherings indoors. They should not be hospitalized, but should be kept in the open air, be well nourished, and given such exercise as will maintain a good physical condition. Occasionally, removal to another climate seems to clear up the condition quickly. They may be isolated in groups, but these groups should not be large, since carriers may develop the disease and infect other carriers, or may harbor meningococci which are virulent for the other individuals.

No reports of satisfactory results in treating the carrier by the use of sprays, swabs, or irrigation have been confirmed.

Gordon's method of the inhalation of chloramine vapors is in the experimental stage, though it has shown some promising results.

Summary.

A routine examination of probable contacts should be made by means of post nasal swabs.

The material can best be collected through the mouth by swabbing the posterior nasopharynx.

The swab should be immediately seeded on a recently prepared solid medium containing sheep serum or laked human, or rabbit, blood in from 5 to 10 per cent strength. The medium should be at body temperature when seeded, and incubated at this temperature for 12 to 20 hours.

To identify the organism, fish the colonies and stain by Gram's method, selecting those colorless, translucent colonies which yield a Gram negative coccus. Subculture such colonies on a slant of the serum agar and suspend the 8 to 18 hour growth in salt solution. Test the agglutination of the suspension against a polyvalent serum at 55° C. overnight.

Continue the identification by sugar fermentation tests on glucose, maltose, and mannose.

Treat carriers by isolation from indoor contact with others and by general hygienic measures.

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[1] Scott, W. M. 1916. Jour. of Hygiene, Vol. 15, No. 3.